Proteins

Body builders and football players eat a lot of protein (eggs, cheese, and meat) to build muscle mass. You have probably seen protein-enriched drinks and protein-enriched foods (power bars) at the supermarket.

Proteins are the most complex and important group of molecules because they possess diverse functionality to support life. Every cell that makes up plants and animals requires proteins for structure and function. Your body and plants also have enzymes. These specialized proteins catalyze chemical reactions that are necessary for metabolism and cell reproduction. Your muscles are made from a variety of proteins, and these proteins allow your muscles to contract, facilitating movement. Other types of proteins in your body are the **peptide hormones**; insulin and glucagon are two common examples.

Proteins are complex polymers composed of **amino acids**. Amino acids contain carbon, hydrogen, nitrogen, and sometimes sulfur and serve as the monomers for making peptides and proteins. Amino acids have a basic structure that includes an amino group (NH_2) and a carboxyl group (COOH) attached to a carbon atom (see **Figure 1A**). This carbon atom also has a side chain (an "R" group). This side chain can be as simple as an -H or a -CH_3, or even a benzene group.

The R groups on an amino acid are analogous to an athlete's clothing and sports equipment. By changing clothing or equipment, an athlete can become more effective as a soccer, football, or baseball player. Although this person is still an athlete, the change can make the athlete more effective in a particular activity or function. The same is true with amino acids. They are still amino acids regardless of the attached R group, but different R groups produce different functions and different properties.

There are twenty amino acids found in the body. Eight of these amino acids are essential for adults and children, and nine are essential for infants. Essential means that we cannot synthesize them in adequate quantities for growth and repair of our bodies, and therefore, must be included in the diet.

Amino acids are linked together by a **peptide bond** in which the carboxyl carbon of one amino acid forms a covalent bond with the amino nitrogen of the other amino acid . Short chains of amino acids are called **peptides**. Longer chains of amino acids are called **polypeptides.** Although the term polypeptides should include proteins, chains with less than 100 amino acid residues are considered to be polypeptides, while those with 100 or more amino acid residues are considered to be proteins.

DON'T FORGET

Many of the major hormones in the body are peptides. These hormones can influence enzyme action, metabolism, and physiology. Insulin, which is given to a person with a specific type of diabetes, is an example of a peptide hormone. Certain antibiotics and a few anti-tumor agents are also peptides. The artificial sweetener aspartame (*Equal*®) is a dipeptide composed of aspartic acid and phenylalanine with a methyl group attached at the carboxyl terminal group (L-aspartyl-L-phenylalanine methyl ester)

The sequence of amino acid residues in a polypeptide chain is critical for biological function. For example, a genetic disease (mutation of a single base pair in DNA) called sickle cell anemia is caused by the substitution of one amino acid (glutamate) with another (valine) in a structural protein called beta-globulin, which is a part of hemoglobin in red blood cells. Hence, a single structural change resulted in a dramatic alteration in physiological function. The ability of an enzyme to catalyze a particular reaction depends on its specific shape. It's a lot like a key and lock—if the key is broken or in a different shape, it won't open the lock. The receptor sites on cell surfaces must be in a specific shape for polypeptide hormones to interact with the cell. With twenty different amino acids and each polypeptide consisting of hundreds of amino acids, it is no wonder that proteins play such a variety of roles in the human body.

DON'T FORGET

Chemistry of Proteins

The protein backbone is formed from the peptide bonds created from the amino and carboxyl groups of each monomer that repeat the pattern -N-C-C- or C-C-N-. The number and sequence of amino acids in a polypeptide chain is referred to as the **primary structure** of a protein. The free amino group and carboxyl group on opposite ends of a polypeptide chain allow proteins to act as pH buffers (resist changes in pH) inside the cell. The amino group (NH_2) accepts a proton and becomes (NH_3^+), and the carboxyl group (COOH) donates a proton and becomes dissociated (COO^-).

As noted previously, each amino acid residue in the polymer may have a different side chain or chemical group attached to it, such as hydroxyl (OH), amino (NH_2), aromatic ring (conjugate rings such as the phenol ring in phenylalanine), sulfhydryl (SH), carboxyl (COOH), or various alkyl (CH_n). This variety of side chain groups on the polymer backbone gives proteins remarkable chemical and physical properties. For example, carboxylate groups can function as carboxylic acids (COO^-), or amino groups can behave as bases (NH_3^+). This allows protein polymers to be multifunctional molecules, with both acidic and basic behavior at the same time! Additionally, the presence of hydroxyls, carboxylates, sulfhydryls, and amino groups allows hydrogen bonding, and the alkyl groups provide hydrophobic interactions, both within the protein polymer itself and between separate protein molecules.

In the case of macromolecules, such as proteins, the polymeric structure of the macromolecule allows it to simultaneously carry many different charges (on different amino acid residues). However, unlike the small single molecules, the amino acid residues are constrained by linear peptide linkages and thus cannot move freely to randomly associate with other charged molecules. Assuming that charged residues will seek to bond with the nearest convenient counter ion, it is most likely that oppositely charged amino acid residues located at different points within a single protein chain will bond. These structural differences result in the folding of proteins into a **three-dimensional structure**, which is, in part, responsible for their functional properties as biocatalysts, structural materials, muscles, and chemical receptors. Proteins can be shaped as long flat sheets or in globular spheres. This leads to the names fibrous or globular for **protein shapes**. Most enzymes are globular proteins.

In standard acid–base chemistry, students learn that molecules carry electrostatic charges based on the type of atoms that make up a molecule and the environment of the molecule. Given that opposite charges attract, cationic and anionic atoms can combine to form covalent bonds, in which electrons are shared between atomic orbitals, or form ionic bonds, in which only electrostatic attractions exist. In solution with smaller molecules, such as HCl (an acid) or NaOH (a base), protein molecules can freely move around and associate with each other on a more-or-less random basis.

Protein polymers extend the simple acid–base charged chemical species concepts to explain how biological systems have greater levels of complexity and can utilize simple, monomeric chemical structures (like amino acids) to create exquisitely complex biological structures like antibodies, muscle, and skin. Protein polymers have physical structure, even when dissolved in liquids. The charged and hydrophobic residues within a protein tend to associate, causing the protein to fold up. When you unfold the protein molecule (called **denaturation**), its charged residues can reassociate with other charged molecules (**precipitation or coagulation**). Protein precipitation is widely used to recover recombinant protein products, enzymes, or in the production of many common foods. Cheeses and soybean tofu are examples of coagulated protein food products.

DON'T FORGET *Enzymes*

Enzymes are protein polymers that possess the ability to specifically "recognize" biological molecules, bind to them, and catalyze a chemical reaction. In contrast to non-protein catalysts, enzymes are specific catalysts—they usually react with only one **substrate**. Since all biochemical reactions are enzyme catalyzed, many different enzymes must exist. An *Escherichia coli* bacterium, one of the simplest biological organisms, has more than 1,000 different enzymes working at various times to catalyze the reactions necessary to sustain life of the bacterium.

The complex molecules that are contained in food provide the energy needed by living organisms to carry out all life functions. These molecules are not useful to the organism unless they are first broken down into smaller, simpler forms through **digestion**. Digestion involves the hydrolysis (breakdown) of proteins to amino acids, starches to monosaccharides, and fats to fatty acids and glycerol. Unfortunately, hydrolysis at body temperature occurs at a rate that is too slow to be useful to the organism. To speed up (catalyze) the hydrolysis reaction, living organisms produce and use enzymes.

Carbohydrate digestion begins in the mouth with an enzyme called salivary amylase. This enzyme is an **alpha-amylase** whose function is to reduce starch, a complex carbohydrate, to simple sugars. Starch is initially reduced to maltose and then to glucose. The glucose is absorbed by the intestines and used to supply energy for the body.

Food Uses of Proteins

Proteins also serve important roles in the processing of food products. They are used for their thickening, gelling, emulsifying, and water-binding properties in meats (sausages), bakery products, cheese, desserts, and salad dressings. Proteins are used for their cohesive and adhesive properties in sausage making, pasta, and baked goods. Egg proteins are used for their foaming properties in desserts, cakes, and whipped toppings. Milk, egg, and cereal proteins are used as fat and flavor binders in low-fat bakery products. Proteins are used for texture and palatability in bakery products (breads, cakes, crackers, and pizza crust) and sausages.

Milk protein consists of 80% casein and 20% whey proteins. There are four major types of casein molecules: alpha-s1, alpha-s2, beta, and kappa. Milk, in its natural state, is negatively charged. The negative charge permits the dispersion of casein in the milk. When an acid is added to milk, the H^+ concentration neutralizes the negatively charged casein micelles. When milk is acidified to pH 4.7, the isoelectric point (the point at which all charges are neutral) of casein, an isoelectric precipitate known as acid casein is formed. Cottage cheese and cream cheese manufacture involves an acid precipitation of casein with lactic acid or lactic acid–producing microorganisms. Acid casein is used in the chemical industry and as a glazing additive in paper manufacturing.

Casein also can be coagulated with the enzyme rennin, which is found in rennet (an extract from the stomach of calves). Rennin works best at body temperature (37^{o}C). If the milk is too cold, the reaction is very slow, and if the milk is too hot, the heat will denature the rennin, rendering it inactive. The mechanism for the coagulation of the casein by the rennin is different from the acid precipitation of casein. The coagulation of the casein by rennin is a two-stage process. In the first stage, rennin (a proteolytic enzyme) splits a specific bond in the amino acid chain of the kappa-casein macromolecule converting it into a para-kappa-casein and a glyco-macropeptide. This causes an imbalance in the intermolecular forces in the milk system, and the hydrophilic (water-loving) macropeptides are released into the whey. Unlike the kappa-casein, the para-kappa-casein does not have the ability to stabilize the micellular structure to prevent the calcium-insoluble caseins from coagulation. In the second stage, colloidal calcium phosphate bridges within the casein micellular structure are formed in the presence of the soluble calcium, resulting in the three-dimensional curd structure. The rennin coagulum consists of casein, whey protein, fat, lactose, and the minerals of the milk, and has a fluffier and spongier texture than the acid precipitate. Rennet is used in the manufacture of cheese and cheese products, and rennet casein is used in the plastics industry.

Casein is solubilized with sodium hydroxide and calcium hydroxide to produce sodium caseinate and calcium caseinate, respectively. Caseinates are added to food products to increase their protein content and are key ingredients in non-dairy coffee creamers and *Cool Whip*®.

Approximately 90% of soybean proteins are classified as globulins, based on their solubility in salts. More specifically, the proteins are conglycinin (a glycoprotein) and glycinin. Tofu is manufactured by coagulating the proteins in soymilk with magnesium sulfate. As bonding occurs between the positively charged magnesium ions and negatively charged anionic groups of the protein molecules, the proteins coagulate.

Activity Objective

In Part 1, you will precipitate casein from milk using an acid. This method is used to make cottage cheese. In Part 2, you will coagulate casein from milk using the enzyme rennin. This method is used for manufacturing cheese. In Part 3, you will coagulate soy protein from soymilk, using magnesium sulfate. This method is used to make tofu.

Materials Required

Distilled white vinegar (acetic acid), 5% acidity
Pasteurized whole milk
Soymilk
Rennet tablets (*Junket*)
Epsom salt (magnesium sulfate)
Cheesecloth
Rubber bands
Stirring rod/wood *Popsicle* sticks
Heatproof gloves
Weigh boats
Hot plate
Beakers
Graduated cylinder
Balance
Thermometer
Foil
Hammer
Eyedroppers
Heatproof pad

Experimental Procedure

Part 1. Precipitation of casein from milk with an acid (vinegar)

1. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of milk in the beaker. Record the weight of the milk in the data table (weight of beaker with milk – weight of beaker = weight of milk).
2. Place the beaker with the milk on a hot plate. Heat the milk to 21^{o}C (70^{o}F). Turn off the hot plate and remove the beaker.
3. Add 11 milliliters (2 teaspoons) of vinegar to the warm milk and stir for 2 minutes, then allow the milk to sit for 5 minutes. The casein will precipitate into heavy white curds.
4. Cut out a piece (2–3 layers) of cheesecloth large enough to cover the top and 2 inches down the sides of a beaker. Using the rubber band, fasten the cheesecloth over the top of the beaker. Pour the curdled milk into the beaker, collecting the curds (casein) in the cheesecloth and allowing the vinegar and whey to drain off into the bottom of the beaker.
5. Gather up the cheesecloth with the casein and rinse in cool water by dipping into another beaker containing water.
6. Squeeze the casein until almost dry, then spread out the cheesecloth to let the casein dry for 5 minutes.
7. Weigh the precipitate. (Do not weigh the cheesecloth with the precipitate). Record your results.

Part 2. Enzymatic coagulation of the casein from milk with rennet

1. Place 1/2 of a crushed rennet tablet into a beaker. To crush the tablet, place it between two pieces of foil and hit the tablet with a hammer.
2. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of milk in the beaker. Record the weight of the milk in the data table (weight of beaker with milk – weight of beaker = weight of milk).
3. Place the beaker with the milk on a hot plate. Heat the milk to 43°C (110°F). Pour the hot milk over the rennet tablet, stir for 2 minutes, and allow the milk to sit on the lab bench for 5 minutes.
4. Collect the curds by pouring the curds and liquid into a beaker covered with cheesecloth (2–3 layers) (see step 4 in Part 1). Gather up the cheesecloth and squeeze out the liquid whey from the curds. Spread out the cheesecloth to allow the curds to dry for 5 minutes.
5. Weigh the curds. (Do not weigh the cheesecloth with the curd.) Record your results.

Variations:
Test the effect of low temperature on the activity of rennet. Repeat the experiment with cold milk at 4°C (40°F). Record your results.

Test the effect of high temperatures on the activity of rennet. Repeat the experiment with hot milk heated to 70°C (160°F). Record your results.

Part 3. Coagulation of protein from soymilk using a salt (magnesium sulfate)

1. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of soymilk in the beaker. Record the weight of the soymilk in the data table (weight of beaker with soymilk – weight of beaker = weight of soymilk).
2. Place the beaker with the soymilk on a hot plate.
3. Bring the soymilk to a boil and turn off the heat. Monitor this step closely; do not allow the soymilk to boil over the top of the beaker.
4. Add 1.6 grams (1/4 teaspoon) of Epsom salt (magnesium sulfate, a mineral salt) to the hot soymilk and stir.
5. Wait until the curds are floating in an almost clear liquid.
6. Fasten a piece (2–3 layers) of cheesecloth over the top of a beaker with a rubber band. Pour the soy curds and liquid into the beaker, collecting the curds in the cheesecloth and allowing the liquid to drain into the bottom of the beaker.
7. Gather up the cheesecloth and squeeze out as much water as possible. Spread out the cheesecloth to allow the curds to dry for 5 minutes.
8. Weigh the curds. (Do not weigh the cheesecloth with the curd.) Record your results.

DATA TABLE – MILK AND SOYMILK CURDS

	Weight of milk/soymilk	**Weight of curd**	**Describe the curd (color, texture)**
Milk + acid			
Milk + rennet			
Soymilk + Epsom salt			

weight of beaker with milk – weight of beaker = weight of milk

Biuret Test

This test is used to indicate the presence of proteins.

Place a pea-size sample of the milk and soymilk curds on a Petri dish. Place 1 milliliter of sodium hydroxide on each curd. Add 5 drops of copper sulfate to each curd. Record the color of the reagent and whether your results are positive or negative.

Repeat the procedure with a potato chip, raw potato, or piece of bread.

DATA TABLE – BIURET TEST ON FOODS

	Biuret test – positive or negative
Milk + acid precipitate	
Milk + rennet coagulum	
Soymilk + Epsom salt coagulum	
Potato chip	
Raw potato	
Bread	

Questions

1. Compare the weights of the curds from the milk (acid and rennet) with that from the soymilk.

2. Why did the casein that was coagulated with the rennet weigh more than the casein that was precipitated with the acid?

3. Compare the amount of acid casein precipitated from the whole milk with the amount of soy protein coagulated from the soymilk. How do your results compare with the Nutrition Facts label for each product?

4. How did the biuret test indicate the presence of proteins?